≥+2; P = 0.002,) and pHER2 (0 vs. ≥+1; P = 0.001) expressions were significantly associated with incidence of EGFR mutation.

Conclusions: EGFR mutation was a significant predictive biomarker of response to gefitinib. Phosphorylated EGFR protein expression is a potent replaceable biomarker for EGFR mutation.

A5-04 **Molecular Targets, Mon, 13:45 - 15:30**

The impact of EGFR mutation and smoking status on non-small-cell lung cancer patients treated with geftinib

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Background: EGFR mutations have been recognized as a predictor of favorable clinical outcomes in non-small-cell lung cancer (NSCLC) patients treated with gefitinib. Never-smoking status is also considered to be a predictive factor and may be a determinant for gefitinib treatment. We have independently reported the relationship between EGFR mutations and clinical benefit in NSCLC patients treated with gefitinib. In this study, we combined our data and re-analyzed the factors that would affect on clinical outcome in patients treated gefitinib.

Methods: The EGFR mutation status restricted in exon19 deletion and L858R exon21 mutation was determined in 408 NSCLC patients who were treated with gefitinib using PCR-based assay. The clinical record including clinicopathological factors, tumor response and survival time were reviewed for 408 patients and correlated with EGFR status.

Results: In the total case, tumor response was observed in 155 patients showed tumor response and 239 patients showed no response. Fourteen patients were not evaluable. Among examined factors, EGFR mutation was only significant factor for tumor responsiveness ($p<0.0001$), prolonged overall ($p<0.0001$) and progression-free survivals ($p<0.0001$) by multivariate analysis. To analyze the impact of EGFR mutation and smoking on treatment outcomes with gefitinib, we classified our patients into 4 groups based on EGFR mutation and smoking status: (A) 104 patients with EGFR mutation and never-smoking history, (B) 64 patients with EGFR mutation and smoking history, (C) 74 patients with EGFR wild-type and never smoking history, (D) 166 patients with EGFR wild-type and smoking history. The survival times including overall survival and progression-free survival of each group were compared (Fig.1). There was no significant difference among A and B groups or among C and D groups. There was significant difference between A or B and C or D groups, confirming that the EGFR mutation not smoking status is a predictor for a prolonged survival of patients (A vs C, $p<0.0001$; A vs D, $p<0.0001$, B vs C, $p=0.0004$); B vs D, $p<0.0001$).

Conclusions: Our large-scale data indicates that EGFR mutation at exons 19 and 21 status is a predictive factor for favorable clinical outcome in patients treated with gefitinib.

A5-05 **Molecular Targets, Mon, 13:45 - 15:30**

Anti-OX40 monoclonal antibody therapy in combination with radiotherapy results in powerful therapeutic antitumor immunity to murine lung cancer

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OX-40, also known as CD134, is a 50-kDa type-I membrane glycoprotein that belongs to the tumor necrosis factor (TNF) receptor superfamily and serves as a T-cell co-stimulatory molecule. *In vivo,* deliberate ligation of OX40 in tumor-bearing mice induced tumor eradication, whereas, in some of the models, administration of agonistic OX40 monoclonal antibody (mAb) alone was not sufficient to induce tumor eradication. Our preliminary experiments also showed insufficient therapeutic outcome using agonistic anti-OX40 mAb alone. Therefore, development of more powerful strategies is definitely required to augment immunotherapeutic effects with agonistic anti-OX40 mAb. Ionizing radiotherapy is one of the core modalities for treatment of localized cancer. However, radiation can cause additional immunosuppressive effects in the cancer host through bone marrow suppression, resulting in reduction of the absolute number of cells responsible for immunity and elevated levels of irradiated tissue-derived TGF-β and IL-10. In contrast, it has been shown that irradiation has a role in enhancing tumor immunogenicity and homing effector cells to the tumor site via induction of tumor apoptosis and upregulation of MHC, costimulatory, and adhesive molecules on tumor cells. Under these circumstances, investigators have attempted to optimize the use of irradiation in concert with various immunological modalities such as recombinant cytokines, cytokine-gene-transduced tumor or virus vaccinations, dendritic cells, agonistic anti-CD40 monoclonal antibody, *ex vivo* activated cells from draining lymph nodes (DLNs), adoptive transfer of antigen-specific T cells and peritumoral injection of CpG ologodeoxynucleotide in mouse experimental models. Based on the previous knowledge, we focused on irradiation as an immunological partner of agonistic anti-OX40 mAb therapy. In the present study, we attempted to elucidate the therapeutic effect of combining agonistic anti-OX40 mAb and irradiation in a murine lung cancer model. After intradermal transplantation of ovalbumin-transfected LLC (LLC-OVA), C57BL/6 mice were locally irradiated with a single dose of 20Gy in combination with intratumoral injection of anti-OX40 mAb at 50μg on day 4 when the inoculated tumor reached a diameter of 7 to 9 mm. On day 8, 11 and 14, the tumor-bearing mice were further treated with the same dose of anti-OX40 mAb. Anti-OX40 mAb in combination with radiotherapy provided greater efficacy than either single treatment against well-established tumors and prolonged survival. *In vivo* depletion study suggested that therapeutic immunity was mainly $CD8^+$ T cell-dependent. $OX40^+CD8^+$ T cells were augmented in draining lymph nodes (DLNs) obtained from irradiated mice compared with those from non-irradiated mice. OVA-MHC tetramer$^+$ $CD8^+$ T cells were highly recruited in DLNs obtained from mice treated with anti-OX40 mAb in combination with radiotherapy,